Progress in Infertility—

Recent Advances in Investigation and Treatment of Infertility

—A Review

Advances in the field of reproductive biology have been so rapid that the traditional approach to the management of infertility is now outdated.

The physician who is concerned with the investigation and treatment of the infertile couple must now be familiar with some aspects of plastic surgery, genetics, immunology, comparative biology and endocrinology. It is the purpose of this review to summarize selectively the more important advances in these areas and to indicate, where possible, their clinical application.

It must be emphasized, however, that a complete history and physical examination of both partners remain the basis of investigation, and the cause for infertility may be determined and be amenable to treatment without recourse to the more sophisticated measures which will be outlined. For this reason the etiological factors which may relate to infertility are summarized in Table I.[52]

It is common practice to divide the program of investigation into two phases. In the first the physician uses easily available office facilities; in the second he admits the patient to hospital to complete the more elaborate investigations with the least expense, inconvenience and delay.

TABLE I.—Etiological factors relating to infertility

Female Factors	*Male Factors*
General	
Dietary disturbances	Fatigue
Severe anemias	Excessive smoking, alcohol
Anxiety, fear etc. (hypothalamus)	Excessive coitus
	Fear, impotence, etc.
Developmental	
Uterine absence, hypoplasia	Undescended testes
Uterine anomalies	Testicular germinal aplasia
Gonadal dysgenesis	Hypospadias
	Klinefelter's syndrome
Endocrine	
Pituitary failure	Pituitary failure
Thyroid disturbance	Thyroid deficiency
Adrenal hyperplasia	Adrenal deficiency
Ovarian failure, polycystic disease	
Genital Disease	
Pelvic inflammation and tuberculosis	Orchitis, mumps
Tubal obstructions	Venereal disease
Endometriosis	Prostatitis
Myomata and polyps	
Cervicitis	
Vaginitis	

Male and Female Factors

Marital maladjustments—Sex problems—Ignorance (timing, douching, sperm leakage, etc.)—Low fertility index —Immunological incompatibility

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UTERINE FACTORS

Intrauterine Adhesions

Renewed interest has been shown in the Fritsch-Ascherman syndrome where intrauterine, and occasionally intracervical, synechiae occur as a result of trauma to the endometrium by the introduction of a foreign instrument.[54] The endometrial cavity is reduced in size, the uterine walls are partially or completely adherent, and the possibility of conception is significantly diminished. Should pregnancy occur, there is a high incidence of abortion, malpresentation, premature delivery and placenta accreta.[14]

The syndrome is characterized by infertility, amenorrhea or menstrual irregularity, and repeated abortion. It has been demonstrated that whenever a foreign instrument is inserted into the uterine cavity bacteria are introduced and remain in all patients for at least 24 hours.[41] There is a particularly high rate of this complication following curettage in the third or fourth postpartum week. Adhesions have developed after removal of hydatidiform moles and as a sequela of tuberculous endometrial infection.

The diagnosis of intrauterine adhesions is dependent on hysterography, for the condition can be recognized by the lacunar pattern. The adhesions may be single or multiple, may vary in size and are central or peripheral in location.[5]

Treatment is primarily surgical, and good results have been claimed following hysterotomy and cervical dilatation from above;[5] from conventional dilatation and curettage followed by intensive broad-spectrum antibiotic therapy;[9] and from curettage and insertion of a plastic intrauterine device followed postoperatively by combined estrogen

and progesterone therapy.[35] Of great interest is the use of tissues transplanted into the uterine cavity, and grafts of fallopian tube, endometrium and fetal membranes have been employed.

In some patients tissue transplantation has been followed by normal menstruation and successful term pregnancy.[53] In view of the high incidence of associated chronic salpingitis with consequent tubal occlusion, tubal patency studies should always be performed before contemplating definitive surgery.

TUBAL FACTORS

Ovum Transport

There is a dearth of information available on the physiology of ovular transport in the human fallopian tube, but work in animals, particularly rabbits and rats, suggests that the effect of estrogen is of considerable importance. There is a transient fall in estrogen following ovulation and the levels rise again a week later. Attempts have been made to correlate the normal decline in postovulatory estrogen with the passage of the ovum along the oviduct.

On the basis of experimental observation in rabbits and mice it may be postulated that moderate elevations in estrogen prematurely expel the ovum into the uterus, or that high levels of estrogen during the critical period may account for the delay in ovular passage and result in tubal pregnancy[20] in the human.

The existence of a tubal-blocking mechanism has also been described, and is probably an important factor in regulating ovum transport. It is believed that this blockage is at the level of the ampullary-isthmic junction. The block appears to be maintained by endogenous estrogen; the postovulatory fall leads to relaxation of the block, and the ova then enter the isthmus. However, equilibrium can be disturbed experimentally by moderate or large doses of estrogen, the former causing premature relaxation of the block and the latter prolonging the duration of delay at the ampullary-isthmic junction.[21]

The role of progesterone in relation to ovum transport is unknown, as is its effect on the tubal block mechanism described above, but the findings of a rich adrenergic innervation of the tubal isthmus leads to speculation about the action of both estrogen and progesterone in catecholamine release in this area.[4]

Tubal Surgery

A critical study of the results of tubal surgery has not, until recently, been encouraging, and despite occasional enthusiastic claims only about 10% of those operated upon eventually achieve a term pregnancy. Over the last decade there has been a gradual increase in the success rate achieved, and the use of plastic surgical techniques, careful preoperative assessment and postoperative management justify a more hopeful attitude. Shirodkar[52] summarized the reasons for the poor results which have come to be expected as inexperience of the surgeon, improper selection of cases and poor techniques. The oviducts are delicate structures, and good results can be obtained only by meticulous techniques and delicate handling of tissues. Only fine suture materials of size 5-0 or 6-0 should be employed and hemostasis is of the greatest importance. The surgeon should use the most delicate instruments available.[32] Before tubal surgery is contemplated the following conditions must be satisfied:

(1) The husband's sperm analysis must be satisfactory. (2) The occurrence of ovulation must be established by endometrial biopsy, hormonal cytosmear and basal temperature readings. (3) There should be no history or clinical evidence of pelvic inflammatory disease occurring within the previous year, and the sedimentation rate and leukocyte count should be normal. Particular attention should be paid to excluding tuberculous disease. The presence of tubal obstruction must be demonstrated by at least three consecutive hysterosalpingograms.

Evaluation of Tubal Patency

Reliance on tubal insufflation alone for the assessment of tubal obstruction may lead to the mistaken impression that there is a pathological occlusion present, when in fact the findings are the result of functional spasm. Hysterosalpingography at the very least should be regarded as a complementary investigation and will outline uterine lesions and localize the site of tubal blockage. Because of the possibility that tubal spasm may mimic pathological occlusion, three successive hysterosalpingograms should be performed, and the site of obstruction confirmed at each screening before a definitive diagnosis is made.

With the advent of cinefluoroscopy and the use of an image intensifier the need has disappeared for using an oil-base contrast medium with its attendant complications of granuloma formation and inspissation of the polymer base which these agents form.[3] However, peritubal and pelvic adhesions cannot be outlined with certainty, and direct visualization by culdoscopy or laparoscopy is necessary.

By this means the pelvic viscera can be examined and tubal patency studies supplemented by injecting indigo carmine dye through an intrauterine cannula. In approximately three out of four patients with an abnormal salpingogram or unexplained infertility, some type of pelvic disorder such as peritubal adhesions or endometriosis is diagnosed at culdoscopy.[54]

Operative Techniques

These include:

(1) Fimbriolysis, the separation of adherent fimbriae which are otherwise normal.
(2) Salpingolysis, the division of peritubal adhesions.
(3) Salpingostomy, the creation of a new tubal ostium.
(4) Resection and anastomosis of a mid-tubal obstruction; and
(5) Tubal reimplantation into the uterus in cases of cornual obstruction.

Most successes are achieved by salpingolysis or fimbriolysis. It is usual to shorten the tubovarian ligaments and perform a ventrosuspension operation by shortening the round ligaments at the same time, and to ensure at the conclusion of the operation that the tubes lie in the pelvis without kinking or stretching.[16] Salpingostomy is employed when distal occlusion of the oviduct has occurred. The occlusion may be associated with dense adhesions involving the ovary, and in these circumstances corrective surgery carries a poor prognosis. More commonly a hydrosalpinx is present and various approaches to its correction have been suggested.

Total linear salpingostomy[24] is rarely practised, and if the incisions are carried medially to involve the narrower segment of the oviduct, proximal tubal occlusion may result.

Various types of and situations for new stomal openings have been suggested, but the most popular method of treatment is resection of the occlusion and reflection of a small cuff of tube to form a new opening. There are those who advocate maintenance of patency with polyethylene or other tubes, and more recently the Rock Mulligan silastic hood has been employed. Many surgeons, however, do not employ these means of maintaining patency, because of the possibility of damage to the epithelium of the tube whose secretory and ciliary activity is vital for ovular transport.[44, 56]

The results from resection and anastomosis of the tube in cases of midsegment obstruction are the worst in tubal surgery. It is often better to resect the segment proximal to the obstruction and reimplant the distal segment than to attempt resection and end-to-end anastomosis.[42] Microsurgical techniques have been applied in this situation, but further experience is necessary before evaluation can be made.

Cornual or interstitial occlusion can be corrected either by reaming or by opening the uterine cavity and reimplanting the open segment of the tube. Polyethylene tubes, rods or stainless steel wires have been used to maintain patency, but here also many surgeons believe that equally good or better results can be achieved without splinting and the introduction of these foreign materials may encourage subsequent infection.[16, 52]

The employment of antibiotics, corticosteroids and antihistamines in the immediate postoperative period is of considerable value in experimental studies,[47] but there are no critical studies available in respect of human tubal surgery, and it appears that simple supportive measures may produce results which are just as excellent. It is certain that postoperative uterotubal lavage or insufflation has resulted in ascending infection which destroys the reconstructive effect of surgery.[16]

Several unusual approaches, which are worthy of mention, are the employment of isografts of gut, appendix and uterine muscle, and homografts of fallopian tubes, but there are no reports of successful pregnancies resulting from these procedures. As a result of advances in tubal plastic surgery the following figures of pregnancy rates can be quoted for the various operative procedures:[16, 44]

Fimbriolysis, 35 to 40%.
Salpingolysis, 35 to 40%.
Salpingostomy, 15 to 25%.
Tubal reimplantation, 35 to 40%.

ENDOCRINE FACTORS

There have been significant advances in knowledge of the biosynthesis and metabolism of ovarian steroids. Information on the pathways of hormone formation, the effects of gonadotropins, cellular sites of steroid synthesis and the considerable number of metabolites is finally forming a logical pattern. The clinical application of this knowledge is not immediately apparent, but as an example it is known that the secretion of androstenedione or dehydro-epiandrosterone in the polycystic ovary syndrome represents normally occurring intermediates, rather than new or abnormal steroids. Many new and more accurate methods are now available for assays of the thyroid, adrenal, pituitary and ovarian hormones. Several excellent reviews on this subject exist.[48, 49, 57]

Defects of Luteal Phase

The significance of the luteal phase defect in relation to infertility and repeated abortions is still under investigation. The diagnosis is made if inadequate luteal phase endometrium is repeatedly found in successive menstrual cycles. The cause is thought to be a deficient production of pituitary interstitial cell-stimulating hormone mediated through hypothalamic failure as a result of a metabolic, nutritional or cytogenic disorder. Persistent defects of luteal phase endometrium are found in 3.7% of all infertile patients[28] and in 35% of infertile patients who have repeated abortions.[27] True primary infertility is rare in these patients, and carefully recorded basal body temperature charts indicate that there is a high incidence of occult abortions.[28] Treatment by replacement therapy is usually satisfactory. If there is an associated estrogen deficiency, combined estrogen and progesterone therapy is indicated. Long-acting progestational agents may be used, as for example 17-alpha-hydroxyprogesterone caproate in doses of 1 to 5.0 mg. per week; while chorionic gonadotropin is effective, it is more expensive to use. The treatment of these patients with clomiphene citrate has usually been unsatisfactory.[26]

Progestins in Habitual Abortion

Adequately controlled studies have demonstrated that progestins and other hormones are of no value in the treatment of habitual abortion.[25, 51] The prognosis in habitual abortion is better than formerly believed, and without treatment 70% or more patients will achieve successful pregnancy. The hormone deficiencies described in patients who abort repeatedly are the result, rather than the cause, of inadequate trophoblastic function, and are often due to a genetically determined abnormality of the conceptus.[22, 51]

Endometriosis

The place of progestational agents in the management of endometriosis is undergoing critical reevaluation. These agents may be used to advantage before and after conservative surgery, in unmarried patients with maximal symptoms and minimal overt disease, and in patients with recurrent disease following a previous conservative operation, who wish to have their reproductive function preserved. Many patients have been found to undergo subjective improvement with objective progression of the process, and gynecologists are placing less and less reliance on the management of this condition by progestin therapy alone.[32]

The Polycystic Ovary Syndrome

It has become apparent that the classical picture of this syndrome first described by Stein and Leventhal in 1935 applies to a small proportion of women with polycystic ovaries. Further, the symptoms may be present with normal or almost normal ovaries, and light and electron microscope studies reveal no

characteristic histological features to distinguish the polycystic ovary from the normal one.[18] Interest is now centred on the ovarian stroma, especially in the medullary region, and it is possible that there may be analogies between the stromal cells here and the "interstitial cells" of animal ovaries.[43]

There is now sufficient evidence to show that the syndrome is characterized by an over-production of ovarian androgens. Plasma testosterone and urinary testosterone glucuronide are both elevated well above normal levels,[8] and since dexamethasone suppression does not alter the higher than normal levels found in these patients it is likely that the site of over-production is in the ovaries.[43]

Two major abnormalities of steroid biosynthesis have been found. One is in the aromatizing system which converts 19-oxygenated androgens to estrogen. The other is in the three B-ol-dehydrogenase system which converts D5-3P-01 to the D5-3-keto configuration. A consequence of the first abnormality is a reduction in the amount of estrogen produced and an accumulation of its precursors (androstenedione and testosterone) due to failure of aromatization, and of the second, that little androstenedione and testosterone are formed from dehydro-epiandrostenedione.[19] The cause of the syndrome remains unknown, but it is probable that the ovarian changes are secondary to abnormalities of pituitary gonadotropin secretion. Abnormalities of the enzymatic system which have been briefly described raise the possibility of genetic factors, but there is no evidence to support this view.[19]

The mainstay of treatment, until recently, has been ovarian wedge resection, but the condition recurs with distressing frequency. Because of this possibility, management in a young woman is problematic and it is reasonable to control symptoms with estrogen-progestin suppression therapy by using a sequential oral contraceptive. Excellent results have been claimed for this regimen.[17] In some series corticosteroids have produced results comparable to those of wedge resection, and there are many advocates of its trial use before operation is undertaken.

Considerable success has been reported with gonadotropins and clomiphene to induce ovulation with subsequent pregnancy in patients with polycystic disease. However, the effect is not permanent, and continued ovulation may depend on continued treatment.[17]

The Induction of Ovulation

In approximately 25% of women who are investigated for infertility, anovulation, either alone or in combination with other factors, appears to be the cause of failure to conceive. Until recently there was little that could be offered to these patients, but the introduction of gonadotropin and latterly clomiphene citrate therapy has altered the situation dramatically, and there is reasonable assurance that when anovulation is the only cause of infertility, a majority of patients can be successfully treated.[13]

CLOMIPHENE CITRATE

Clomiphene citrate is a relatively simple chemical compound allied to chlorotrianisene (Tace). It is a non-steroidal compound possessing no estrogenic, progestogenic or androgenic properties. Its mode of action is thought to be by stimulation of the hypothalamo-pituitary axis to release gonadotropins, and following its exhibition a rise in the excretion in the urine of follicle-stimulating hormone, and later of luteinizing hormone, can be demonstrated. Some investigators maintain that its primary effect is on the ovary where, by competitive inhibition, the drug acts as an anti-estrogen, reducing the inhibitory effect on the pituitary of the naturally occurring estrogens.[40]

Clomiphene is suitable only for use in the treatment of infertility associated with anovulation and is not indicated in the treatment of such menstrual disorders as menorrhagia or oligomenorrhea, or of patients with endometrial hyperplasia.[48,49,57] A considerable proportion of patients with primary amenorrhea have chromosomal aberrations such as are found in Turner's syndrome, or anatomic defects such as congenital absence of the uterus. None of these can be expected to respond to clomiphene, and primary amenorrhea should be thoroughly investigated before clomiphene therapy is undertaken.

It is in the area of anovulation associated with secondary amenorrhea that clomiphene therapy finds its most appropriate application. In carefully selected patients the success rate approaches 70%, but this can be achieved only after the selection made possible by complete preliminary workup. Women with endogenous ovarian activity sufficient to ensure fairly regular menstruation but who do not ovulate are the most rewarding group, but studies such as assays of urinary pregnandiol and urinary estrone excretion are necessary to determine whether a patient can expect a successful outcome from the therapy. If these methods of investigation are not available, the occurrence of withdrawal bleeding following the exhibition of progesterone will constitute a valuable clinical test for adequate endogenous estrogen.

A satisfactory response to clomiphene therapy is indicated by such signs of ovulation as biphasic basal body temperature, a progestational pattern in the vaginal smear, the disappearance of ferning in cervical mucus, or a urinary pregnandiol excretion of over 2 mg. per 24 hours. Endometrial biopsy is contraindicated, as it may disturb a pregnancy.[13]

Management.—If the patient has regular, spontaneous or induced uterine bleeding, treatment should commence on or about the fifth day of the cycle and 50 mg. of clomiphene should be given daily for five days. The dose should be increased to 100 mg. in subsequent courses if no response is obtained. The majority of patients who are going to respond will do so in the first course of therapy, and three courses constitute a normal clinical trial, but up to six courses in consecutive months may be given before it can be concluded that the patient is a "clomiphene failure". Ovulation usually occurs from between 6 and 12 days after completion of the course, and properly timed coitus is therefore of the greatest importance. In patients classified as having "hypothalamic amenorrhea" with low total gonado-

tropins, low estrogens in urinary assay and smooth ovaries at culdoscopy, ovulation may often be induced by treatment with 100 mg. of clomiphene for 10 consecutive days followed by one injection of 10,000 international units of human chorionic gonadotrophin four days after the last dose of clomiphene.[33]

Clomiphene is contraindicated in patients with liver disease. Liver function tests should be carried out in every patient with a history of liver disease or jaundice before administration.

Side effects are few; hot flushes, abdominal discomfort, ovarian enlargement and visual blurring have been reported. Ovarian enlargement and cyst formation are more likely with high or prolonged dosage. For this reason patients should have a pelvic examination before each course of clomiphene is given.

Results.—Between 60 and 75% of the patients treated have an ovulatory response and about 40% become pregnant. The incidence of spontaneous abortion is high, in the region of 20%, and that of multiple pregnancy about 8%. In patients with polycystic ovary syndrome the chance of ovulatory response from wedge resection and clomiphene is equal. The Chiari-Frommel syndrome, characterized by postpartum amenorrhea and galactorrhea, has shown a gratifying response to clomiphene therapy, although supplementation with chorionic gonadotropin is occasionally necessary.[13, 33]

GONADOTROPIN THERAPY

The main indications for the treatment of anovulatory infertility with gonadotropins are low or absent total gonadotropin excretion, and failure to induce ovulation by clomiphene citrate. Patients with primary amenorrhea should be investigated in order to exclude pituitary or hypothalamic tumours and non-pituitary causes, as, for example, thyroid or adrenal dysfunction.

In order to induce ovulation, gonadotropin with follicle-stimulating and luteinizing activity must be used. Follicle-stimulating hormone (FSH) is now obtained from the urine of postmenopausal women and is known as HMG. Most experimental experience has been gained with Pergonal, which has in the main an FSH activity with only a slight luteinizing effect, insufficient to stimulate progesterone secretion. Its action is therefore supplemented with luteinizing hormone (LH) derived from the urine of pregnant women (HCG).[45]

Management.—There is a considerable variation in response, and therapy must be carefully individualized. An accurate assessment of follicular activity can only be obtained by measurement of total estrogen excretion (normal: 20 to 60 μg. per 24 hours). Ferning of cervical mucus and changes in spinnbarkeit can only be rough estimates, and values vary depending on individual interpretation. High levels of estrogen excretion above 100 μg. per 24 hours are associated with ovarian enlargement, ascites and the occurrence of multiple pregnancies. Evidence of ovulatory response can be obtained from basal temperature charts, pregnandiol excretion assays and vaginal cytology. Therapy is initiated by the daily intramuscular administration of two ampoules of HMG (containing 150 to 160 i.u. of FSH). Pelvic examination should be performed at every visit, beginning on the fourth or fifth day of treatment. If there is a good follicular response, one ampoule of HMG is given daily for a further two days, followed by one injection of 800 to 1200 i.u. of HCG. If no ovarian enlargement occurs but there is evidence of an ovulatory response, a second cycle should be induced. Dosage schedules are then adjusted in relation to individual responses to therapy.[55]

Results.—Ovulation can be induced in over 90% of patients treated and about 65% of these become pregnant. The abortion rate is about 25% and multiple pregnancies occur in about 14% of patients.[35]

MALE FACTORS

There has been a concentration of interest in those factors in the male which may contribute to an infertile marriage. Although routine investigation includes examination of sperm morphology, volume, abnormal forms and motility, no two laboratories are agreed on standards of normality and in many cases opinions vary widely among technicians working in the same laboratory.[38, 39]

Work is proceeding to establish base lines of normality and abnormality which will gain wide acceptance, and the employment of electronic cell counters and newer staining techniques are yielding valuable information.[38] Stimulation of spermatogenesis in patients with chronic oligospermia has received considerable attention. A wide variety of hormones, vitamins and other substances has been used, to little or no avail.

The "rebound" phenomenon following testosterone administration has not been of sustained value, but in cases of hypogonadotropic eunochoidism, gonadotropins have been shown to be helpful. In this limited area HMG (Pergonal) and HCG therapy have restored normal sperm production.[38] The place of clomiphene in male infertility is under investigation.[29] Acquired obstructive disorders involving the epididymis, vas deferens, ejaculatory ducts or urethra can now be treated surgically with a good measure of success. Epididymovasostomy is successful in 40% of cases where there is epididymal obstruction only and spermatogenesis is normal. Vasovasostomy is performed where reversal of previously ligated vasa efferentia is indicated. The technique is simple and a high proportion of patients are made fertile.[7]

IMMUNOLOGICAL CONSIDERATIONS

The possible role of immune mechanisms in infertility is of current interest. Immunoelectrophoretic studies of seminal plasma have demonstrated the presence of specific proteins. Human semen contains at least 16 antigens, and spermatazoa seven, of which four are common to seminal plasma.[46] There is good evidence for believing that the antigens in the testes and spermatozoa are responsible for initiating an immunological response, and are located in the acrosome.[12]

The intracutaneous injection of homologous testicular homogenate results in severe damage to the germinal epithelium of the testes

within two to three weeks, and the effective antigen is a polysaccharide-polypeptide complex.[6] When this male antigenic material is introduced into the female genital tract, a classic immunological response occurs. There is a primary response followed by a secondary response, resulting in the over-production of antibodies. Detection of these antibodies forms the basis for the test of Franklin and Dukes.[15] Recent work has shown that there is a heavy deposition of tissue antibodies at the site of maximal antigen exposure, namely the vagina, cervix and lower uterine segment.[50] Among the many possible biological effects is the development of a hypersensitivity state which may be responsible for the expulsion of semen at coitus and failure of sperm migration to the fallopian tubes.

Clinical Implications

To test this hypothesis a series of infertile couples, in which the female had a positive antibody reaction, was treated by having the male use a condom at intercourse. The majority of patients became antibody-negative within 2 to 12 months, and 54% subsequently became pregnant. Although there are many valid criticisms in regard to selection of the patients investigated, the results are an indication that an immunological mechanism may play a part in infertility. It is pertinent to enquire why all females, or at least a percentage, do not become "immunologically infertile". It is possible that the female may possess enzymatic systems capable of degrading the antigens produced under most circumstances, or that the situation may parallel Rh disease in that some women have a genetically inheritable trait.[30]

Investigators are now interested in the possibility of an immunological basis for spontaneous abortion and, in particular, habitual abortion, in which the antigen is feto/paternal in origin.[1] There is as yet insufficient evidence available to evaluate this aspect of infertility, but the field of immunoreproduction is only in its formative stages and is attracting the interest of an increasing number of workers.[2]

CYTOGENETIC FACTORS

Sex Chromatin

Nuclear sex chromatin ("Barr" body) is a valuable marker for the presence of two X chromosomes, and in man corresponds to the presence in the cell of the major proportion of one X chromosome. The number of sex chromatin bodies is equal to the number of X chromosomes present minus one. Multilobed polymorphonuclear leukocytes also have a sex-specific marker in the "drumstick", present as a nuclear appendage in female patients. In normal females between 30 and 60% of buccal cells will have sex chromatin bodies, and between 2 and 3% of polymorphs will have a "drumstick". It is now suggested that if a discrepancy is found between the counts in the buccal cells and the polymorphs this is an indication that mosaicism may be present.[37]

Sex chromatin examination is indicated in all newborn infants with ambiguous genital development (adrenogenital syndrome), in all cases of delayed puberty (Turner's syndrome and testicular feminization), in cases of gynecomastia (Klinefelter's syndrome) and in all cases of female and male infertility. There are a number of situations under present investigation, among them the determination of nuclear sex from cells recovered from the amniotic fluid for genetic counselling in inherited disease.

Aberrations of Chromosome Constitution

The importance of chromosome analysis can be understood when it is realized that one in 300 newborn infants has some type of chromosomal abnormality affecting growth, and that in at least 12.5% of all spontaneous abortions chromosomal errors can be demonstrated. Extrapolating these figures it has been conservatively estimated that the overall frequency of abnormal chromosome complements at the early developmental stages of embryos is 1.5% or more. Aberrations of chromosome constitution are not infrequent, and to take examples related to infertility, 40% of patients with primary amenorrhea will be found to have such an aberration. Because the sex chromosomes determine the development of the sex organs, abnormalities of these chromosomes are of particular interest in infertility investigation.

The etiology of chromosomal error is not understood. Down's syndrome (trisomy 21) is related to maternal age; it is thought that most errors arise during chromosome meiotic division, due to faulty segregation (non-disjunction or anaphase lagging), although there is some evidence that post-zygotic mitotic non-disjunction may be of importance because of the frequency with which mosaic individuals are found.[34] Such rearrangements of chromosomes as translocations, inversions and deletions may be caused by external agents, for example by virus infection, radiation or mutagenic drugs, but it is obvious that most mechanisms are at present unknown.

Turner's Syndrome

The syndrome characterized by short stature, infantilism, streak ovaries, congenital lymphedema, webbing of the neck and occasionally coarctation of the aorta is associated with a 44 XO constitution. However, many are mosaic with an XO/XX, XO/XXX or XO/Xx isochrome karyotype. There is a correlation between the cytogenetic and phenotypic abnormalities that are found, and the occasional XO/XY mosaic may show histological evidence of spermatogenesis. XO may be lethal and a number of spontaneous abortions have this chromosomal constitution. The reason for the exertion of a lethal effect in some instances, but not in others, remains unknown. The treatment indicated in XO/XY individuals may be endocrine substitution therapy and plastic surgery.[10]

Klinefelter's Syndrome

This syndrome is manifested by cryptorchidism, azoospermia and gynecomastia, and the individual possesses a 44 XXY chromosome constitution. Individuals have been identified with up to five X chromosomes, but the potent Y chromosome is usually associated with tubular differentiation and external male characteristics.

As mental retardation is progressively related to an increasing number of X chromosomes, a large number of patients with Klinefelter's syndrome have to be sent to institutions for the mentally retarded. A number of mosaics have been described and examples are XY/XXXY; XO/XY/Xx/XxY and XY/XXY; 1D:D translocation.

The presence of two or more Y chromosomes has been of interest recently, and many individuals with this chromosome constitution are tall in stature and are high-grade mental defectives with aggressive behaviour patterns and criminal records. While their genital development is normal, little is known of their fertility.[11]

Hermaphrodites

True hermaphrodites by definition have gonadal tissue of both sexes which may be separate or combined in either or both gonads. All degrees of abnormality and intermediate development of phallus and labioscrotal folds may be seen and a rudimentary phallus may be present. Breast development and body hair distribution are extremely variable.

Menstruation may occur from the vaginal orifice, or periodic hematuria may occur from the vaginal orifice in those patients in whom the vagina communicates with the bladder. A confusing variety of cytogenetic findings have been reported. Most patients have a 44 XX karyotype, but a remarkable number of mosaics have been described, ranging from XO/XY to XX/XXY/XXYYY.[36]

Pseudohermaphrodites

Male pseudohermaphrodites possessing testes and external female genitalia are characteristic of the testicular feminization syndrome. They have cryptorchid or inguinal testes and a small vagina, and internal female genitalia are absent. Breast development is normal, pubic hair is sparse and primary amenorrhea the rule. Almost all have an XY karyotype. Female pseudohermaphrodites are most commonly the results of virilization by familial adrenal hyperplasia and have a normal 44 XX chromosome complement.

Amenorrhea

Primary amenorrhea is a symptom in many patients with the syndromes described above. Many other cytogenetic anomalies are associated with irregular or scanty menstruation, and this may occasionally be found in the "super female" where more than two X chromosomes are found, although a large number of these patients are normal in every respect. Up to five X chromosomes have been reported.[31]

Spontaneous Abortion

The association between spontaneous abortion and aberrations of chromosome number and probable constitution has been amply demonstrated. The most commonly found aberrations are trisomies for both autosomes and sex chromosomes, monosomy of sex chromosomes, mosaics and triploidies. Most chromosome anomalies are found in abortions from the first trimester of pregnancy, and the more severe the aberration the earlier the conceptus is aborted.

A number of slighter aberrations, more particularly translocations, are being intensively investigated by means of family studies. It is likely that further progress will be delayed until large series are investigated and more refined techniques of chromosome examination are available.[23]

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